

We claim:

1. A process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:
 - (a) determining the level of a membrane transport protein at the plasma membrane of the cell using a method comprising:
 - (i) contacting the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein at the plasma membrane of the cell; and
 - (ii) determining the level of ligand bound to the membrane transport protein;
 - (b)
 - (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein; and
 - (c) comparing the level of ligand determined at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the level of the membrane transport protein inside the cell.
2. The process according to claim 1 wherein the membrane transport protein is a glucose transport (GLUT) protein.
3. The process according to claim 2 wherein the membrane transport protein is GLUT4.
4. The process according to claim 3 wherein the GLUT4 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.
5. The process according to claim 2 wherein the membrane transport protein is GLUT1.

6. The process according to claim 5 wherein the GLUT1 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 12.
7. The process according to claim 1 wherein the membrane transport protein is a mutant membrane transport protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.
8. The process according to claim 7 wherein the reduced rate of recycling or transporter internalization of the mutant membrane transport protein increases the level of the mutant membrane transport protein at the plasma membrane of a cell compared to the level of a wild-type form of the membrane transport protein.
9. The process according to claim 8 wherein the mutant protein is a mutant GLUT4 protein.
10. The process according to claim 10 wherein the mutant GLUT4 protein comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9.
11. The process according to claim 1 wherein the membrane transport protein is labeled to facilitate binding of the ligand to the membrane transport protein.
12. The process according to claim 11 wherein the label comprises one or more copies of a peptide, polypeptide or protein that is heterologous to the membrane transport protein.
13. The process according to claim 12 wherein the label comprises one or more copies of a peptide, polypeptide or protein selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β -galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-

shifted green fluorescent protein (SEQ ID NO: 29), cyan fluorescent protein (SEQ ID NO: 30), biotin, streptavidin, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 20, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 21, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 31 and mixtures thereof.

14. The process according to claim 13 wherein the label comprises influenza virus hemagglutinin (HA) (SEQ ID NO: 15).
15. The process according to claim 12 wherein the label is positioned within an extracellular domain of the membrane transport protein.
16. The process according to claim 15 wherein the label is positioned within the first extracellular domain of a GLUT protein or a mutant thereof.
17. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT4 protein or a mutant GLUT4 protein that comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.
18. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT1 protein that comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 13.
19. The process according to claim 1 wherein the cell is a eukaryotic cell.
20. The process according to claim 19 wherein the cell is a mammalian cell
21. The process according to claim 20 wherein the cell is a cell selected from the group consisting of a 3T3-L1 fibroblast cell, a 3T3-L1 adipocyte cell and a C2C12 cell.
22. The process according to claim 1 wherein the ligand capable of binding to the membrane transport protein is an antibody.

23. The process according to claim 22 wherein the antibody is a monoclonal antibody.
24. The process according to claim 23 wherein the monoclonal antibody is an anti-hemagglutinin (HA) tag antibody capable of binding to an amino acid sequence set forth in SEQ ID NO: 15.
25. The process according to any one of claims 22 to 24 wherein the antibody is labeled with a detectable marker selected from the group consisting of an enzyme label, a radiolabel and a fluorescent label.
26. The process according to any one of claims 23 to 25 wherein the antibody is labeled with a fluorescent label.
27. The process according to claim 1 wherein the plasma membrane is permeabilized or disrupted by contacting the plasma membrane with an agent that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur.
28. The process according to claim 27 wherein the agent that permeabilizes or disrupts a membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β -D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α -hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof.
29. The process according to claim 28 wherein the agent that permeabilizes or disrupts the membrane is saponin.
30. The process according to claim 1 wherein the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds to the ligand for a time and under conditions sufficient for an antibody-antigen complex to form and determining the level of the complex wherein the level of the complex indicates the level of the ligand bound to the membrane transport protein.

31. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using an assay selected from the group consisting of immunofluorescence, immunohistochemistry, and an immunosorbent assay.
32. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using a fluorescence linked immunosorbent assay.
33. The process according to claim 1 additionally comprising providing the cell expressing the membrane transport protein.
34. The process according to claim 33 wherein providing the cell expressing the membrane protein comprises transforming or transfecting the cell with an expression construct that encodes the membrane protein.
35. The process according to claim 1 additionally comprising fixing the cell.
36. The process according to claim 35 wherein the cell is fixed prior to or at the same time as permeabilizing or disrupting the plasma membrane of the cell.
37. The process according to claim 35 or 36 wherein the cell is fixed with a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol and glutaraldehyde.
38. The process according to claim 35 or 36 wherein the cell is fixed with formaldehyde.
39. The process according to claim 1 additionally comprising inducing translocation of the membrane transport protein to the plasma membrane.
40. The process according to claim 39 wherein inducing translocation of the membrane transport protein to the plasma membrane comprises contacting the cell with an amount of one or more peptides, polypeptides, proteins or compounds sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur.

41. The process according to claim 40 wherein the cell is contacted with an amount of sucrose and/or an amount of insulin sufficient to induce translocation.
42. The process according to claim 41 wherein the cell is contacted with sucrose and/or insulin in the presence of serum.
43. The process according to claim 1 additionally comprising inducing resistance to translocation of the membrane transport protein in the cell.
44. The process according to claim 43 wherein the membrane transport is a GLUT protein or a mutant GLUT protein and wherein inducing resistance to translocation of the membrane transport protein in the cell comprises contacting the cell with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient for resistance to insulin induced translocation to occur.
45. The process according to claim 44 wherein the cell is contacted with insulin in the absence of serum.
46. The process according to claim 45 wherein the cell is contacted with insulin for between about 24 hours and about 48 hours.
47. The process of claim 1 comprising:
 - (a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:
 - (i) contacting a cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein;
 - (b) determining the level of the membrane transport protein within another cell using a method comprising:
 - (i) permeabilizing or disrupting the other cell;

- (ii) contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;
 - (iii) determining the level of ligand bound to the membrane transport protein; and
 - (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.
48. The process according to claim 47 wherein the cells are isogenic or from the same cell line.
49. The process according to claim 47 or 48 wherein the cells are cultured under substantially similar conditions.
50. The process according to claim 49 wherein the level of the membrane transport protein at the plasma membrane of the cell and the level of membrane transport protein within the cell are each determined in a plurality of cells.
51. The process according to claim 50 additionally comprising normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of ligand bound to the membrane transport protein is determined.
52. The process according to claim 51 wherein the number of cells is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a cell or component thereof for a time and under conditions sufficient for binding of the antibody or ligand to the cell or component thereof and determining the level of antibody bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of the number of cells.
53. The process according to claim 52 wherein the ligand is wheat germ aggluti

internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

- (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(b) determining the level of membrane transport protein within another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

- (i) permeabilizing or disrupting the other cell;
- (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- (iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

55. A process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell, wherein the cells are contacted with insulin in the absence of serum and wherein

the cells are contacted with insulin for a period of time from about 24 hours to about 48 hours;

(b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell at (a) using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(c) determining the level of labeled GLUT4 protein or labeled mutant GLUT4 protein in another cell at (a) but not (b) using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

56. A process for determining the level of recycling of a membrane transport protein in a cell or a change in the level of recycling of a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process according to any one of claims 1 to 54, wherein the other cell is cultured for a longer period of time than the cell at (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to thereby determine the level of recycling of the membrane transport protein in the cell, wherein a change in the level of the

membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

57. A process for determining a mutation in a nucleic acid encoding a mutant membrane transport protein that is capable of modulating translocation of said membrane transport protein, said method comprising:

- (i) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54; and
- (ii) determining the level of the wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54,

wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that is capable of modulating the level of level of translocation of the membrane transport protein to the plasma membrane.

58. A process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

- (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to any one of claims 1 to 54;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process according to any one of claims 1 to 54, wherein a difference in the level of the membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.
- (c) optionally, determining the structure of the candidate agent;
- (d) optionally, providing the name or structure of the candidate agent; and
- (e) optionally, providing, the candidate agent.

59. A process for determining a candidate compound for the treatment of insulin resistance comprising:

- (a) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a

candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(e) optionally, providing, the candidate agent.

60. The process of claim 59 wherein the insulin resistance is associated with diabetes.

61. The process according to claim 60 wherein the diabetes is type II diabetes.

62. A process for manufacturing a medicament for the treatment of insulin resistance comprising:

(a) determining a candidate compound for the treatment of insulin resistance using a process comprising:

(i) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the

level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

- (b) optionally, isolating the candidate agent;
- (c) optionally, providing the name or structure of the candidate agent;
- (d) optionally, providing the candidate agent; and
- (e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.